CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The clinical and basic aspects of hepatic encephalopathy (HE) will be reviewed.

The pathogenesis of HE and the corresponding therapeutic modalities based on will be outlined, while the role of GABA/BzR complex and BzR ligands will be discussed in detail separately.

Previous clinical experimental trials with flumazenil on HE patients will be summarized and discussed.

2.2 Definition and Epidemiology of HE

As we know, hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome characterized by a global depression of CNS function, which may progress to impaired consciousness and coma. This syndrome is one of severe complications of hepatocellular function failure[1,2].

2.2.1 Types of HE

Depending upon the underlying hepatic diseases, their duration, and degree of hepatic dysfunction, HE may present as one of two major types[2,3,4]. These two types of HE have different clinical manifestations and prognosis.

The most common form of HE, **portal-systemic encephalopathy (PSE)**, occurs most frequently as a complication of chronic liver disease (cirrhosis) and increased portal-systemic shunting. When an acute episode of HE occurs in this setting, it is commonly associated with one or more recognizable precipitating factors. Chronic HE or PSE tends to be a milder, more persistent and more episodic variant of the syndrome that occurs in patients with chronic liver disease and appreciable portal-systemic shunts. The term "subclinical" HE has been more emphasized recently[4,16] which is applied to patients with chronic liver disease who do not display overt behavioral, neurological, or electroencephalograph (EEG) changes but who have abnormal scores in psychomotor tests. These changes are much more reversible by conventional management of HE.

Fulminant hepatic failure (FHF) is another type of HE when the encephalopathy complicates acute hepato-

cellular failure, and the total duration of liver disease is less than 8 weeks at the onset of encephalopathy. Mortality of 85% or higher are typical, and there is usually little confusion in differentiating these patients from those with encephalopathy due to chronic liver disease. Since FHF patients will not be enrolled in this study, this entity will not be discussed in detail.

2.2.2 Precipitating Factors

Well recognized precipitating factors[2,3,4] include constipation, infections, an oral protein load, hypokalemic metabolic alkalosis and other electrolyte and acid-base disturbances, diuretic therapy, diarrhea, vomiting, hypoglycemia, hypoxia, anemia, hypotension, abdominal paracentesis, dehydration and hemorrhage into the gastro-intestinal tract. Any sedative-hypnotic drug can precipitate or exacerbate encephalopathy in patients with poor hepatocellular function. It should be noted that sometimes, it is difficult to detect certain precipitating factors. As liver disease progresses, patients appear to become more susceptible to the adverse effects of circumstances likely to precipitate HE[3,7].

The distribution of causes (more precisely, precipitating factors here, noted by the author) of HE

hypertension. Furthermore, a disproportionately large fraction of the patients who develop severe PSE are derived from a subgroup who have bled from varices and who have then had portal-systemic shunts. Overall, about 8-10% of cirrhotic patients have one or recurrent episodes of severe encephalopathy in their disease course, while the mild or subclinical HE may occur much more often.

Since most B-type hepatitis patients will gradually and unavoidably progress to be cirrhotic within 10 to 20 or more years, it is deemed to be a serious problem for the patients themselves, their families and the society, if the complications of cirrhosis (e.g., esophageal variceal hemorrhage, HE, ascites, etc.) breakout or appear. Most of the cirrhotic patients suffer from the complications at the age of 30-60 years old, therefore the lost or decreased productivity is obvious. Patients with encephalopathy are a much heavier burden in the sense of lost of social communication and depression of the emotion of the people around. Treatment for them is necessary[5].

from 100 consecutive patients reported by Fessel and Conn[17] (1972) is shown in Figure 2.1. In that series, 71% of these episodes were produced by nitrogenous substances from the gut and then the systemic circulation.

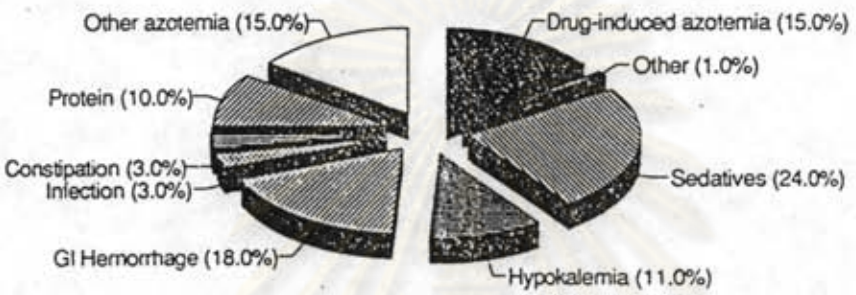

Figure 2.1 An Analysis of the Cause of 100 Consecutive Cases of HE[17]

2.2.3 Epidemiology

The epidemiology of PSE (HE) has not been studied in detail[2,5]. It can best be considered within the context of cirrhosis, since patients who develop PSE are almost invariably cirrhotic. They come from the subgroup of cirrhotic patients who develop portal hypertension and portal-systemic shunts. Approximately 80% of patients with clinically overt cirrhosis have evidence of portal hypertension, as shown by the presence of ascites and of esophageal varices, the prime clinical indices of portal

2.2.4 Situations of Research Settings

Shanghai area has a high prevalence of Hepatitis B Virus (HBV) infection, and now Hepatitis C Virus (HCV) infection rate is increasing, which will also lead to cirrhosis ultimately. Though prevention of the infection of these hepatitis viruses should be set as the priority, because of the lack of effective methods and common knowledge about the risks, new cases still increase. Moreover several effective treatments, like interferon[3] therapy, which can block or slow-down the progression of cirrhosis, are not available for all the chronic hepatitis patients because their high cost. Some cost-effectiveness studies are being designed in Shanghai. No results have come out yet.

It is found that, like the other areas, hemorrhage in the gastrointestinal tract because of the rupture of esophageal varices is the most common complication of the cirrhotic patients[3], which likely progresses to certain degrees of HE. History of porto-systemic (porto-caval, porto-mesenteric, or spleno-renal, etc.) shunting operation also tends to make HE occur much more easy and episodic. The research setting, Zhong Shan Hospital, a national-level general hospital affiliated to Shanghai Medical University, has a strong Division of

Gastroenterology and Hepatology and Institute of Liver Cancer. The patients suffering from liver diseases are from East China and even from other domestic areas or overseas. Thus, it is not unusual to find the cirrhotic patients with HE in the Emergency Room, General Surgical Wards and Digestive Disease Wards, though most of them are mild. Unfortunately no epidemiological study has been successfully accomplished so far.

2.3 Clinical Manifestations and Diagnosis of HE

The clinical features of HE include a broad spectrum of fluctuating psychiatric and neurological abnormalities[1,2,3]. These features depend on the nature and intensity of etiologic and precipitating factors. However, individual variability is obvious. Moreover, encephalopathy of other causes, such as failure of the renal, respiratory and cardiovascular function, hypoglycemia or sedative overdose, can mimic the nonspecific manifestations of HE[2,3,5].

That's why the diagnosis of HE must be made on the basis of clinical criteria, and differential diagnosis is a must[5]. Although there are laboratory tests that are helpful; there are, as yet, no definitive tests for this condition.

2.3.1 Medical History

Detailed medical history[1,2] obtained either from the patient or, preferably, from his family members or colleagues and previous doctors should be the first step for assessing the cirrhotic patients with known or suspected HE, focusing on the elements that define the nature, severity, and duration of the liver disease; the evolution of symptoms of disturbed cerebral function; and an identification of factors that may have precipitated the current episode[5].

2.3.2 Physical Examination

General physical examination that follows should not only concentrate on the vital signs and the presence of liver disease, but should also include a search for features of other disorders that might mimic HE[5].

Although the fetor hepaticus[1,18] is often regarded as one of the components of the HE (PSE or FHF) syndrome, it is not always present in that disorder. It may, however, sometimes, be present in non-hepatic disorders. It seems to vary in intensity and odor quality from patient to patient and from time to time. Of course, its detection depends greatly on the experience of the

doctors, moreover, there is no way to quantify it. Nevertheless, it can give the doctors some hints.

2.3.3 Mental Status Examination and Grading

The practical, simplest but also absolutely important initial assessing method for the different severity of HE is to grade HE by conducting the **mental status examination**[2,3,16]. A great deal of attention should be focused on the aspect of this examination, which includes 3 components of examination -- the state of consciousness, intellectual function and personality-behavior.

- **Disturbed consciousness** with disorder of sleep is usual, like hypersomnia, insomnia or inversion of sleeping pattern. Slow responses, a fixed stare or apathy and disorientation may appear. Semistupor may progress to stupor and complete unresponsiveness (deep coma).

- **Personality changes** include exaggerations of normal moods, attitudes, or behavior. Childish, euphoria, irritability, anxiety or depression may appear. Sometimes patients

may exhibit inappropriate even bizarre behavior.

- **Intellectual deterioration** varies from slight impairment of computation to gross confusion. Some psychometric tests may not be successfully accomplished. Degree of forgetfulness may progress until totally losing himself.

The **clinical stage of HE**, reflecting the severity of HE, is evaluated mainly by the **Grade of Mental Status of HE**[2,3].

Mental status is assessed using the West Haven criteria for the grading of HE (Conn et al)[2] as the following (Table 2.1):

Table 2.1 Grade of mental status of HE

Grade	Contents
Grade 0	No abnormality detected;
Grade 1	Trivial loss of awareness, euphoria or anxiety, shortened attention span, impairment of addition or subtraction;
Grade 2	Lethargy, disorientation for time, obvious personality change, inappropriate behavior;
Grade 3	Somnolence to semistupor, responsive to stimuli, confusion, gross disorientation, bizarre behavior;
Grade 4	Coma, tests of mental function not possible.

(Details of the grading shown in Appendix 1)

Though this grading is widely accepted and applied, a drawback is its inability to accommodate the detection or quantitative assessment of patients with subclinical or mild HE[16].

2.3.4 Psychometric Tests

The simplest psychometric tests[2,16] used in the assessment of HE include orientation to time, person, and place, recall of current and remote events, the subtraction of serial 7's, handwriting, and figure drawing. Although simple to administer, these tests either lack precision, or cannot be easily quantified, and are only useful to detect relatively gross neuropsychiatric defects. One more precise but simple and also easily-quantified test is known as the **number connection test (NCT)** (Figure 2.2), which has been extensively evaluated by Conn et al[2]. Many previous clinical trials[6,19,20,21,22] of lactulose, lactitol and branched-chain amino acid on HE adopted NCT as a major measurement tool. It is recommended that this test be administered to all patients with mild HE as part of their routine clinical assessment[16] It is the relative changes, other than the absolute length or degree, of the time period accomplished by certain patient that represent the real stage and changes of intellectual

action and mental status more appropriately. Chronic and mild, subclinical or suspected HE patients are more proper candidates. Age-adjusted NCT has also been investigated[23]. To avoid or minimize the effect of learning from serial testing, different variations of the test of equal difficulty have been designed, and is recommended[11,37].

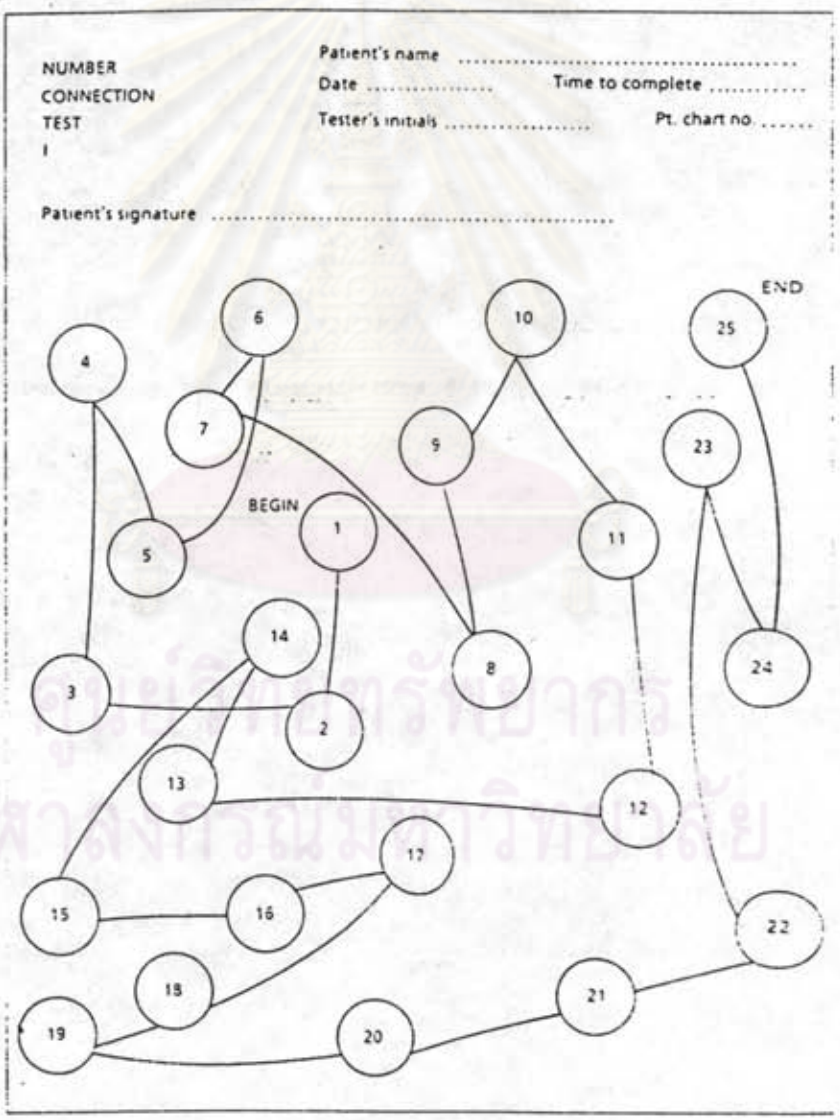

Figure 2.2 Number Connection Test (NCT) [3]

2.3.5 "Flapping" Tremor -- Asterixis

The most characteristic neuromuscular abnormality is the "flapping" tremor (asterixis)[2]. However a "flapping' tremor is not specific for HE. This sign may be elicited from patients with a variety of metabolic or structural abnormalities, like uremia, respiratory failure or congestive heart failure[5]. Since axterixis is not in the entity of mental status but of a neuromuscular abnormality, it seems logical to be assessed independently as an assistant factor[1,16].

2.3.6 Laboratory Tests

No single laboratory test is useful[5,16] in assessing HE. The main value of most laboratory tests in patients with is to aid in the differential diagnosis of encephalopathies or to detect precipitating factors of HE. They must be performed at the outset and some should be repeated serially.

2.3.6.1 Child-Pugh Scores and Stages

The liver functional status of a patient with suspected HE should be evaluated. The most common and practical method of this evaluation is to exam the Child-Pugh Stage[24] (Grade or Score) (Table 2.2), which converts

the levels of 5 components into different scores to form a scale. The higher the score, the poorer the liver function and the more severe the liver disease. The Child-Pugh Grade has also been widely used as a prognostic index for the liver diseases[1,3].

Table 2.2 Child-Pugh sore and grade

Clinical & Biochemical Measurements	Points Scored for Increased Abnormality		
	1	2	3
Bilirubin (mg/dl)	1 - 2	2 - 3	> 3
Albumin (g/dl)	3.5	2.8 - 3.5	> 3.5
Prothrombin time (seconds prolonged)	1 - 4	4 - 6	> 6
Ascites	Absent	controllable	uncontrollable
Encephalopathy	None	1 and 2	3 and 4

Grade A score 5 or 6; Grade B score 7, 8 or 9; Grade C score 10 to 15

2.3 6.2 Blood Ammonia Concentration

The value of measuring blood ammonia levels in patients with HE has remained controversial for many years[5]. Although some correlation may exist between the blood ammonia level and the severity of HE[2], the correlation is often poor and in an individual patient is uncertain. It should be emphasized[16] that the signs and symptoms of HE may precede an increase in the blood

ammonia concentration; conversely, a raised blood ammonia concentration is not specific for HE; and blood ammonia levels may not sensitive enough to be useful in monitoring the effects of treatments on HE. It might rather be taken as part of the overall assessment of patients with HE[2,16].

2.3.6.3 Other Tests

Other laboratory tests[16] that have been suggested as being useful in assessing HE include measurements of the serum levels of certain substances which are the proposed pathogenic factors for HE, like phenols, mercaptans, amino acids, short-chain fatty acid and GABA. However most of these tests are not available for the routine lab work.

Lumbar puncture for cerebrospinal fluid (CSF) test and computed tomography (CT) scans for cranial changes are the two tests that are useful in the differential diagnosis of a patient with an encephalopathy, but are of only minimal value in the assessment of HE[5,25].

2.3.6.4 Electrophysiological Tests

Electrophysiological studies[2,5], particularly visual evoked responses (VERs) and the

electroencephalogram (EEG), may help, in certain degree, in the diagnosis, differential diagnosis and management of the patients with known or suspected HE, though mild. The simple EEG assessment of a patient in HE is to grade the degree of abnormality of the conventional EEG trace using a scheme such as that proposed by Parsons-Smith et al or revised one by Conn and Lieberthal[2]. There is a fairly good correlation between the degree of abnormality of the EEG and the clinical stage of HE. Controversy still exists. It has also been found that the EEG changes associated with this syndrome are not specific for HE. Therefore the changes must be explained with caution. Serial recordings and computer assisted analysis are recommended.

2.3.7 PSE Index

Since none of the individual tests used in the assessment of HE patient is an entirely satisfactory index of HE by itself, an arbitrary composite index of the degree of HE is derived, when the results of such tests are expressed quantitatively and combined. A practical index, proposed by Conn et al[2], termed the **PSE Index**, can readily be derived from data routinely generated in the assessment of HE patients. It includes 5 weighted components (Table 2.3), among which the mental

status (the clinical grade), the most important of the components, contributes most to the Index.

Table 2.3 Components and weighting factor of PSE Index

Components	Weighting Factor
Mental status	3
NCT	1
Asterixis	1
Blood ammonia level	1
EEG	1

The value of this Index has been demonstrated in not a few clinical trials[6,19,20,21,22]. It should be noted that this Index is particularly recommended to be applied in the evaluation of the chronic HE patients. Appendix 2 gives the details of conducting this Index.

2.4 Pathogenesis and Therapeutic Modalities of HE

2.4.1 Proposed Pathogenic Mechanisms

2.4.1.1 Basic Pathophysiological Abnormalities

It is clear that a normally functioning liver is necessary to maintain normal brain function[3]. Basic and clinical experiments show that, to fulfill this function, the ability of the liver to remove certain substances

from plasma may be more important than its ability to deliver other substances to the systemic circulation. Accordingly, HE may be due primarily to failure of the liver to metabolize adequately certain substances in plasma which modulate CNS functions (Figure 2.3).

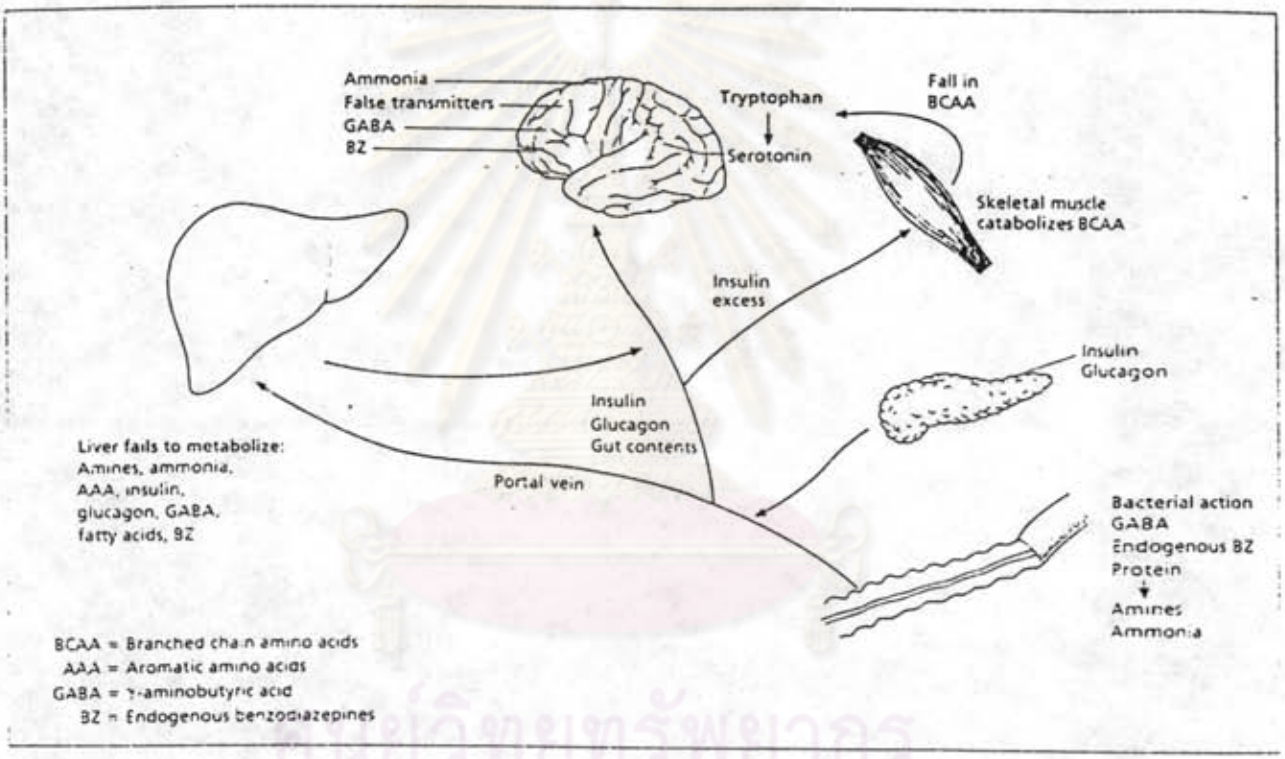

Figure 2.3 The possible factors concerned in the pathogenesis of HE[31]

These substances may accumulate in the systemic circulation, due to the decrease in hepatic extraction and metabolism of these gut-derived nitrogenous substances and the increase in extra- or intra-hepatic porto-systemic shunts in the acute or chronic hepatic insufficiency or failure[2,3,5]. In some cases (e.g., FHF) hepatic insufficiency predominates, whereas in others (e.g., congenital porto-caval shunts) vascular rearrangement predominates. However, the degree of hepatic insufficiency and vascular shunting lies between these two extremes in most cases of HE.

2.4.1.2 The Role of Blood-Brain Barrier

Alteration in the permeability of the blood-brain barrier (BBB) have been hypothesized[1,5] to contribute to the pathogenesis of HE. Recent studies[26] indicate that BBB permeability is nonspecifically increased in models of FHF. Thus the development of HE in FHF may involve an increase in BBB permeability leading to an enhanced transfer across the barrier and the accumulation in the CNS of neuroactive metabolites. It is less certain that a major change in BBB permeability occurs in chronic liver failure.

2.4.1.3 Neuroactive Agents

The identity of these neuroactive substances has been the hot point in the field of hepatology and psychoneurology for a long time, and, however, still remains controversial. Such substance (Figure 2.3) relevant to the pathogenesis[22,3,4] of HE should be (a) nitrogenous, (b) of enteric origin, (c) synthesized by gut flora and/or present in the diet, (d) found in the portal circulation, (e) metabolized by the normal liver, and (f) able to cross the BBB in liver failure and affect CNS function. Elevate or otherwise abnormal concentrations of a large number of substances are found in the plasma, CSF, and brains of animal models and patients with HE[3,27]. These findings have led to the formulation of several hypotheses of the pathogenesis of HE, none of which has been conclusively validated. Therefore, a conservative and conventional view is that "almost certainly, the etiology of HE is multifactorial".

2.4.1.4 Several Mechanisms

During the past four decades, following the development of appropriate animal models of HE and following studies of clinical HE patients and using postmortem brain tissue from them, elevated levels of

ammonia and certain amino acids have been widely advocated as playing major roles in pathogenesis of HE (Figure 2.3) Other substances, such as mercaptans, free fatty acids, and phenols, have also received extensive consideration. Most recently, GABA and BzR agonists have been proposed as prominent factors in the evolution of HE[1,4,12].

Table 2.4 Hypothetical pathogenesis of HE

- Ammonia neurotoxicity
- Multiple neurotoxins and their synergistic actions
  - * Mercaptans
  - * Short-chain fatty acids
  - * Phenols
- "False" neurotransmitters
  - * Octopamine
  - * Phenylethanolamine
  - * Decreased ratio of concentrations of BCAA/AAA
- Neurotransmitters
  - * Serotonin
  - * Catecholamines (dopamine, noradrenaline)
  - * Excitatory amino acid neurotransmitters (glutamate, aspartate, etc.)
  - * GABA and GABA-Bz receptor complex (details see later)

Rather than decreased brain energy metabolism, neurotransmission failure is considered as the cause of

HE. Therefore, current theories on the pathogenesis of HE focus on the origin of neurotransmitter abnormalities which could be responsible for the neural inhibition characteristic of this syndrome. These substances, their concentration changes and their hypothetical roles on the pathophysiological mechanisms of HE[2,4,8] could be pragmatically summarized in Table 2.4.

Multiple reviews have discussed sophisticatedly about this complicated field.

2.4.2 Therapeutic Modalities

Consequently, the therapeutic modalities for the management of HE are based on these hypothetical pathogenesis. In fact, in the history of HE study, the researches of therapies and pathogenesis have been mutually integrated and achieved[4,5].

2.4.2.1 General Principles for Treating HE

Table 2.5 General principles for treating HE

⇒ General supportive measures
⇒ Withdrawal or correction of any potential precipitating factors
⇒ Reduction of absorption of nitrogenous substances
⇒ Treatment of complications of liver diseases
⇒ Improvement or maintenance of hepatocellular function
⇒ Reduction of portal-systemic shunting
⇒ Reversion of neuropathophysiological events directly (if possible)

2.4.2.2 Certain Modalities

Certain medical modalities for treating HE are listed in the following table (Table 2.6), some of them can be cooperatively prescribed[2,4,5]:

Table 2.6 Therapeutic modalities

- Prescribed diet
  - ⇒ to maintain nitrogen balance
  - ⇒ to prevent protein-calorie malnutrition
- Antibiotics -- mostly, neomycin
  - ⇒ to reduce the production, then the absorption of ammonia and other nitrogenous toxins of bacterial origin by changing colonic flora and with suppression of the action of selective species
  - ⇒ be cautious for its ototoxicity and nephrotoxicity
- Lactulose and related carbohydrates (lactitol, etc.)
  -- a combination of cathartic and metabolic actions
  - ⇒ to reduce the time available for toxin absorption from the gut by its laxative effect
  - ⇒ to promote increasing ammonia nitrogen fixation and incorporation into bacterial protein, with a resultant decrease in urea and ammonia production
- Branched-chain amino acid supplement
  - ⇒ to correct the decrease in plasma BCAA/AAA ratio
  - ⇒ to improve the nutritional status
- Miscellaneous therapies
  - ⇒ Biochemical conversion of ammonia to less toxic forms
    e. g., glutamic acid, aspartic acid, arginine, etc.
  - ⇒ Enemas
  - ⇒ Dialysis
- Benzodiazepine receptor antagonists (see later)

2.5 The Role of GABA/BzR Complex in Pathogenesis and Treatment of HE

2.5.1 History and Logistics

The syndrome of HE is marked by profound neuroinhibition, the major manifestations of which are similar to those associated with increased GABA-mediated neurotransmission.

Schafer and Jones (1982) first[12] proposed that an increase in CNS GABAergic neurotransmission involved in the pathogenesis of HE. This increase could be effected by several mechanisms. These include changes in one or more of the proteins constituting this receptor complex or increased concentrations of ligands which bind to this complex[4]. This latter mechanism is consistent with several lines of clinical evidence indicating that humoral factors of gut origin may be precipitants of HE[3].

2.5.2 Organization and Function of GABA Receptor Complex

The receptor of GABA, the principle inhibitory neurotransmitter in the brain, should be more precisely termed as $GABA_A$ receptor complex (Figure 2.4).

This complex[6,8,17] has been traditionally subdivided on a pharmacological basis into a $GABA_A$ receptor, a BzR, and a chloride ionosphere (which is thought to contain sequences that recognize barbiturates) located on the post-synaptic neural membrane. Activation of the $GABA_A$ receptor (by GABA or GABA mimetic) opens the chloride ionophore (channel), increasing neuronal membrane permeability to chloride ion (Cl^-). The entering of Cl^- of the cell effects a hyperpolarization, resulting into the increased GABAergic tone, the following inhibitory effect appears. Humoral factors that could change GABAergic tone, besides GABA itself, include the ligands for the receptors on the complex which potentiate the action of GABA, and the concentration change of these substances.

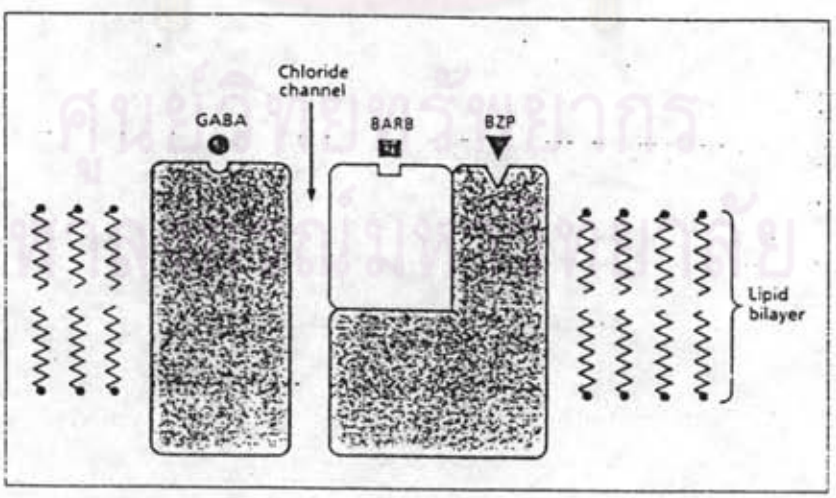

Figure 2.4 Simplified model of GABA receptor complex Embedded in a Post-synaptic Neural Membrane[3]

The basic function[4,29] of the BzR is to allosterically modulate GABA receptor gating of the chloride channel. Occupation of the BzR by an agonist (e.g., diazepam, midazolam) increase the frequency of GABA-gated Cl^- channel opening and the affinity of GABA for its receptor. The principle pharmacological (i.e., sedative, myorelaxant, anxiolytic, and anticonvulsant) properties of BzR agonists are mediated through this mechanism. In contrast, "inverse" agonists of BzR (e.g., DMCM*) possess proconvulsant, convulsant, and anxiogenic actions due to their ability to decrease GABA-gated Cl^- conductance by decreasing the frequency of GABA-gated chloride channel openings. Antagonists of the BzR (e.g., Ro 15-1788 and Ro 14-7437) lack or have minimal (agonist or inverse agonist) intrinsic activity over a wide concentration range. They do not alter the frequency of chloride channel openings but compete with agonists and inverse agonists for binding sites on the BzR. Thus, through this competitive antagonistic effect, the BzR antagonists are able to modulate the GABAergic tone indirectly[4,29] -- tend to normalized changes in GABAergic tone induced by agonists or inverse agonists.

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* DMCM methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate

2.5.3 Evidence of GABA/BzR complex and BzR agonists involved in HE

Implication of GABA/BzR complex in the mediation of HE was originally suggested by finding that the abnormal patterns of VERs in animal models of FHF were similar to those induced by drugs which increase GABAergic tone[14]. Other studies also gave the electrophysiological, neurochemical, and behavioral evidence suggesting that GABAergic tone is increased in these models of HE. That Bz agonists may contribute to the manifestation of HE was strongly suggested by unequivocal transient behavioral and electrophysiological amelioration of encephalopathy in rabbits with galactosamine-induced FHF by the Bz antagonist flumazenil. This finding was shown to be neither species nor model specific. Direct evidence of Bzs (BzR agonists) contributing to the neuronal depression is their presence in the models of this syndrome and the HE (PSE and FHF) patients as well, moreover their levels are elevated than normal[7,25,30]. Recent research[27] extends the earlier studies by demonstrating that increasing blood levels of BzR agonists, so-called as "endogenous benzodiazepines"[30], correspond to worsening of HE. Thus, the ability of the antagonists to reversibly increase neuronal activity in HE is best explained by the

displacement of an agonist from the BzR. It was excited to note that BzR antagonists could substantially correct both the electrophysiological and behavioral manifestations of HE in several animal models and patients with acute or chronic liver failure[7]. These findings indicate that the BzR ligand concentrations are sufficiently elevated to produce some of the behavioral changes of HE. The origin of these Bzs present in HE is not clear. It appears unlikely that some Bzs are synthesized de novo in mammalian. Alternatively, diazepam and N-desmethyldiazepam could be produced by prokaryotes in the gut. Another possibility is that these compounds may be dietary in origin. Regardless of their origin, BzR agonists should be sufficiently extracted and metabolized by normal liver[4]. However, the failure of accomplishing this task, accompanied by the hypersensitivity to Bzs in the condition of liver severe dysfunction, these substances would augment GABAergic tone, thereby contributing to the development of HE[10].

2.5.4 Theoretical Basis of BzR Antagonists on HE

Theoretically there are two properties[10] of BzR ligands that could lead to increase neuronal activation and amelioration of manifestations of HE. One would be

the ability to displace agonists from the BzR. The other would be an intrinsic inverse agonist action that should result in an analeptic effect. However, the full inverse agonist, DMCM, in subconvulsive doses, was not effective in ameliorating HE in an animal model. In particular, it induced a preconvulsive state but did not efficaciously reverse the behavioral manifestations of HE. Thus in HE a full inverse agonist is contraindicated. Conversely, full agonist, which would compound encephalopathy, is also contraindicated. However, compounds classified near the center of the spectrum (Figure 2.5) of BzR ligand activities can subtly and incrementally modulate GABA-mediated neurotransmission through the BzR.

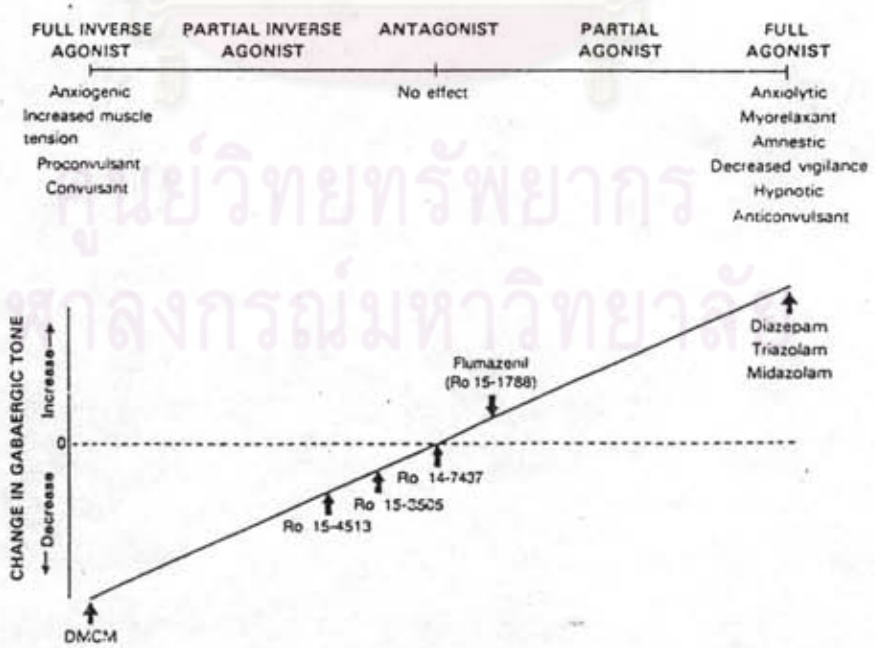

Figure 2.5 Spectrum of activities of the central Bz receptor Ligands[7]

Therefore, flumazenil, the BzR antagonist, emerges likely to have a promising future in the field of HE.

2.5.5 Pharmacokinetical and Pharmacological Properties of Flumazenil

The imidazobenzodiazepine flumazenil (Ro 15-1788, Anexate™, Roche) (Figure 2.6) is a selective, high-affinity, competitive antagonist of the BzR[4,26]. It can be rapidly and almost completely absorbed and extensively distributed through the body when administered orally and intravenously. The amount of binding to plasma protein is relatively low. The plasma clearance (Cl_p) is rapid in normal humans as is the plasma $t_{1/2}$ (45.7 ± 8.5 min., mean ± SD). However, the Cl_p decreases and the $t_{1/2}$ increases to 75 - 142 minutes[31] in patients with cirrhosis. Cerebral levels of flumazenil decrease with a half-time of 25-38 min., which can also be prolonged in the cirrhotic patients. The bioavailability through the oral route is low. It is extensively metabolized by the liver into 3 inactive metabolites and completely eliminated within 48-72 hours in the urine. It is usually administered intravenously.

situations involving the reversal of the sedative and hypnotic actions of BzR agonists[4]. Studies clearly indicate that flumazenil is clinically efficacious in reversing all of the neurological effects of BzR agonists, with rapid onset action and almost no adverse effects[26]. Given the data from basic science studies of animal models and humans with HE indicating that BzR agonists involve in the pathogenesis of HE, it is logical to assume that flumazenil, a BzR antagonist, may be efficacious in ameliorating some of the manifestations of HE in man[13]. In fact, on the other hand, it is such experimental therapies that present the evidence of and support such hypothetical pathogenesis of HE. These studies, most of which are uncontrolled trials, have shown some characteristics and advantages of this drug in the clinical management of HE.

2.5.7 Advantages of Flumazenil on HE

One of the distinct advantages of flumazenil[4,32] over the other conventional therapies is its **rapid onset of action** within minutes (from 28 seconds to less than 60 minutes)[33,34], while the conventional therapies mediate their effects one day or more after the administration. There is a natural tendency to expect that when the diagnosis of HE is made, improvement should follow the

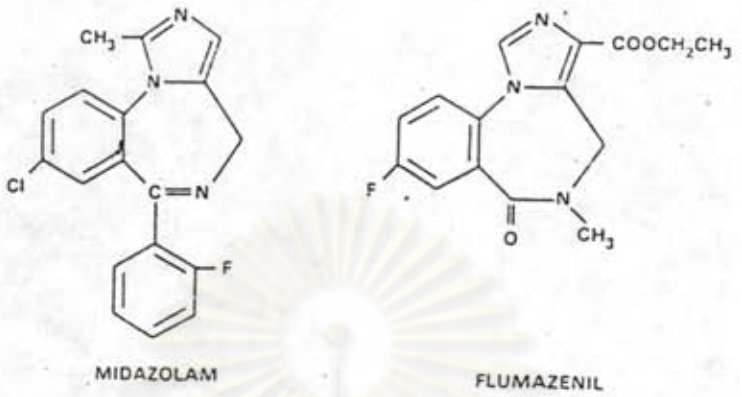

Figure 2.6 The Chemical structures of central BzR ligands[27]:Midazolam - acts as an agonist Flumazenil - acts as an antagonist

Numerous in vitro and in vivo binding studies indicate that flumazenil competitively displaces BzR ligands. The binding is unaffected[4,7,10,26] by the presence of GABA, pentobarbital, and Cl^-. Unlike GABA receptor antagonists, BzR inverse agonists, and chloride channel blockers, flumazenil is not a convulsant and has been found to lack significant intrinsic activity of any type[26], and to be free from any significant side effects, being well tolerated[4].

2.5.6 Potential Clinical Use of Flumazenil

The therapeutic use of flumazenil is presently restricted in Europe and to relatively special clinical

therapy as soon as possible, especially the mental arousal reaction. The fast and effective improvement of mental status of HE patient[3,5] is particularly important to the patients and their relatives in terms of reconstruction of the communication between the patient and the surrounding and the alleviation of the anxiety of the family members. Such a reaction could also help the physicians to obtain the information of the diagnosis and treatment much more directly from the patients. Moreover it could decrease nurses' workload on the patient with bizarre and uncooperative behavior and help the physicians avoid using regimen of sedatives[2,3,4] (usually Bzs, which are quite often prescribed in the routine work) to control the patient's impaired neuropsychiatric behavior because such regimen may aggravate the problem of mental impairment according to the hypothetical pathogenesis. Clearly flumazenil would not influence any underlying liver disease. Combinations of different approaches based on different mechanisms are recommended in the management of HE patients. However, the early and fast-onset effect of flumazenil could reduce the exposure of HE patient to the enhanced GABAergic tone, which would consequently be expected to have potential impact on the restore and maintenance of brain function and to

influence, in some degree, the survival rate combined with other therapeutic interventions[5,10].

Index of prognosis[4,18] is another advantage of flumazenil administration. Some previous trials implied that such favorable neurological response to BzR antagonists probably indicates that the encephalopathy is uncomplicated and potentially reversible[18,34,35]. In some sense, this would help the physicians select and plan their case management scheme. Further research should be done for the selection of patients who might give positive response to the drug as well as adjustment of its dosage and proper route of administration and finally evaluation of clinical effectiveness.

Moreover, the **toxicity** of flumazenil[25,31,32] is **minimal**. Except for some anxiogenic properties, no significant adverse reactions have been observed. Flumazenil can be readily administered orally, which is useful in outpatient management by increasing protein tolerance to optimize liver function and improve nutritional status, and ultimately to make the quality of life better[36].

At last, flumazenil could be prescribed as an assistant diagnostic tool to **differential diagnosis**[4,37] of

severe encephalopathy (coma) of benzodiazepine intoxication, HE or other causes.

2.5.8 Discrepancies and Problems to be Resolved

However, there are some problems which need to be resolved[27]. So far, there is no convincing laboratory evidence that GABA/BzR complex and its neurotransmission involve in the pathogenesis of all kinds of HE, especially of PSE. Clinical trials, even controlled ones, with BzR antagonists have yielded beneficial results in not a majority of cases and it is unclear whether these beneficial effects are due to the action of the action on "endogenous" or pharmaceutical Bzs previously ingested by these patients.

Pharmacokinetical studies of flumazenil[26,31] showed its short elimination half-time, but the variety of the maintenance of the improvement effect induced by flumazenil in the previous studies[13,14] was also noted that some HE patients showed the stable maintenance of improved mental status after the administration of flumazenil, some didn't respond to the drug or maintained the improvement only from less than one hour to several hours. It is considered[4] that differences of these duration are to be influenced in different extent by the flumazenil administration dosages and methods and the HE

patients' own conditions as well. And the effects of other concomitant therapies for HE could not be totally excluded. Unfortunately, the exact relationships among these factors and the minimal effective dosage of flumazenil and generally-accepted administration method have not been set up so far.

In addition, the effect of early-used flumazenil in the long-term integrated systematic management of HE hasn't been well studied and has been found difficult to conduct because of the tendency of spontaneous remission of the manifestations of HE and the ameliorative effects induced by standard therapies after several days of intervention based on the ethical concerns. So far it is unclear whether standard therapy induces a better outcome than flumazenil[4].

2.6 Review Of Flumazenil In Clinical Trials

Previous (from 1985 to 1995) uncontrolled and controlled trials (Ref. 18, 29, 32, 33, 35, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 and 52) indicated that flumazenil may improve HE (FHF and PSE), clinically or electrophysiologically or both, in certain or a subgroup of HE patients. These reports are listed in the following 3 tables (Tables 2.7, 2.8, 2.9).

Table 2.7 Flumazenil: in open clinical trial via iv (intravenous) route

Investagors[a]	Etiology[b]	No. of cases	No. of episodes	Bz screened	Initial grade	Dosage	First react time[e]	Lasting time	No. of responders	No. of posi episodes	Results
Van der Rijt, et al 1995 <49>	FHF & CI	13	13	most posi[c]	NA[d]	1 mg iv bolus	NA	NA	3 (clin) 2 (EEG)	same	no effect
Van der Rijt, et al 1989 (abstr) <48>	FHF & CI	9	9	most posi	NA	10 mg iv bolus	NA	NA	2 (clin) 2 (EEG)	same	no effect
Bansky G, et al 1989 <18>	CI	14	14	2 posi	II - IV	0.2 - 9.6 mg iv	n mins	1-2 hrs	10	10	71%
Ferenci P, et al 1989 <36>	PSE (shunt)	1	2	nega	I - III	1 mg iv bolus	30 secs	NA	1	2	100%
Pidoux B, et al 1989 <16>	CI	7	7	NA	NA	NA	NA	NA	6	6	85%
Grimm G, et al 1988a <41>	CI	8	9	nega	I - IV	2 - 15 mg iv (?)	15 mins	120 mins-No wors[f]	6	6	75%
	FHF	9	11	nega	III-IV	2- 15 mg iv (?)	3 mins	35 mins - No wors	6	6	66%
Burke A, et al 1988 (letter) <33>	CI	1	2	nega	IV	0.5 mg iv bolus	28 secs	1-2 hrs	1	2	100%
Grimm, G, et al 1988b (abstr) <35>	CI	6	6	NA	NA	NA	NA	NA	6	6	100%

(to be continued in the next page)

Table 2.7 (continued) Flumazenil: in open clinical trial via iv (intravenous) route

Investagors	Etiology	No of cases	No of episodes	Bz screened	Initial grade	Dosage	First react time	Lasting time	No of responders	No of posi episodes	Results
Sutherland L, et al 1988 (letter) < 47 >	FHF	1	1	nega	IV	05-3 mg iv bolus	no effect	no effect	0	0	no effect
Meier & Gyr 1988 (abstr) < 34 >	PSE & CI	3	5	NA	II - III	bolus + iv +po (large)	< 60 mins	NA	3	5	100%
Grimm G, et al 1987 (abstr) < 42 >	FHF & CI	5	5	NA	NA	NA	NA	NA	4	4	80%
Bansky G, et al 1985 (letter) < 38 >	CI	4	4	nega	III-IV	02-8 mg iv	40 secs	1-2 hr	2	2	50%
Scollo-Lavizzari G & Steinmann E 1985 (letter) < 46 >	FHF	1	1	nega	IV	05 mg iv	1 min	1 hr	1	1	100%

a The format is: Author(s), Published year, (Publication type-originals unless indicating letter or abstract), Reference No.< >
b Etiology of HE: FHF = fulminant hepatic failure, CI = Cirrhosis, PSE = Porto-sysmetic encephalopathy
c posi = positive, nega = negative
d NA = non-available
e time: mins = minutes, secs = seconds, hrs = hours
f no wors = no worsening

Table 2.8 Flumazenil: in open clinical trials via oral route

Investagors[a]	Etiology[b]	No. of cases	No. of episodes	Bz screened	Initial grade	Dosage[d]	No. of responders	No. of posi[e] episodes	Adverse effects	Results
Seebach J 1992 (letter) < 50 >	CI (PBC)	1	1	NA[c]	III-IV	(iv first) 25 mg bid po × 48 hrs	1	1	obvious: anxious, restless, disoriented, etc	effect
Ferenci P, et al 1989 < 36 >	PSE (shunt)	1	repeated	NA	I-IV	(iv first) 25 mg bid po × months	1	NA	none	effect
Meier & Gyr 1988 (abstr) < 34 >	FHF & CI	3	5	NA	II - III	(iv first) 30 mg qid po × 3 days	3	5	none	effect

a The format is: Author(s), Published year, (Publication type-originals unless indicating letter or abstract), Reference No.< >
b Etiology of HE: FHF = fulminant hepatic failure, CI = Cirrhosis, PBC = primary biliary cirrhosis, PSE = Porto-sysmetic encephalopathy
c NA = non-available
d Dosage: iv = intravenous, po = per oral route, bid = twice a day, qid = four time a day, hrs = hours
e posi = positive

Table 2.9 Flumazenil: in controlled clinical trials

Investagors[a]	Design[b]	Etiology[c]	No. of cases[d]	No. of episodes	Bz scre-ened[e]	Initial grade	Dosage	First reac time[f]	Lasting time	No. of responders	No.of posi episodes	Results
Cadranel JR, et al 1995 < 39 >	R-DB	CI	14	18	NA	NA	0.4-1 mg iv bolus	4 mins (EEG) 30 mins (clin)	NA	NA	EEG F: 12 P: 0	p<0.01
Van der Rijt, et al 1995 < 49 >	DB-CO	FHF & CI	18	//	nega	NA	iv bolus + drip	NA	NA	F: 6 P: 2	//	p=0.06
Pomier-L G, et al 1994 < 45 >	R-DB-CO-PlaCtrl	CI	F: 11 P: 10	F: 13 P: 15	2 resp (+) 2 non-resp (+)	IV	2.0 mg iv bolus	5 mins	45-240 mins or no wors[g]	F: 6 P: 0	F: 6 P: 0	p<0.05
Gyr K, Meier R, et al 1993 (draft) < 51 >	R-DB-PlaCtrl	CI PSE	F: 22 P: 15	//	nega	I - III	2.2 mg iv bolus	immed	NA	F: 6 P: 0	//	p=0.076
El Younsi M, et al 1991 (abstr) < 40 >	PlaCtrl	NA	F: 18 P: NA	//	NA	NA	NA	NA	NA	F: 13 P: NA	//	p<0.05
Van der Rijt, et al 1989 (abstr) < 22 >	DB-CO	FHF & CI	8	//	nega	NA	iv bolus + drip	15 mins	NA	F: 3 P: NA	//	no diffe.
Klotz U. & Walker S. 1989 (letter) < 43 >	R-DB-CO	CI	2	2	nega	III	1 mg iv bolus	no effect	no effect	0	//	no diffe.

a The format is: Author(s), Published year, (Publication type-originals unless indicating letter or abstract or draft), Reference No. < >
b Design: R = randomized, DB = double-blinded, CO = cross-over, PlaCtrl = placebo controlled
c Etiology of HE: FHF = fulminant hepatic failure, CI = Cirrhosis, PSE = Porto-sysmetic encephalopathy
d Cases: F = flumazenil group, P = placebo group
e Bz screened: NA = non-available, nega = negative, resp = responder, (+) = positive
f Reaction time: mins = minutes, secs = seconds, hrs = hours, clin = clinically, EEG = electroencelophagraphically
g no wors = no worsening

It should be noticed:

1) Because of the literature's availability, not all the articles have been reviewed by the author (me) directly, therefore some data unavoidably are adopted for the other reviews or even missing.

2) These trials included several pieces of case report.

3) Some reports were published in abstract form, which are unavailable for the detailed information.

4) Some studies enrolled PSE and FHF patients in the same group, which might not reflect the exact reaction pattern to flumazenil.

5) The eligibility criteria, the initial grade of HE, the administration dosages and methods, and the evaluation measures are not consensus, which, thus, leads to different interpretation of these results; in addition

6) From the point of view of the author (me), several researches have enrolled a batch of same patients, the data of which were used in

the articles published or presented in different time or different journals.

Re-inspection of these articles is necessary, other than simply summing the results up.

In 1991, a review[13] which included the uncontrolled trials up to that date showed that 46 episodes of HE in 41 patients had been treated with flumazenil with a short term success rate of 72% (33/46). According to the etiology of HE, 31 episodes in 28 patients associated with chronic cirrhosis had a success rate of 74% (23/31), while 15 episodes in 13 patients with fulminant hepatic failure has a success rate of 66% (10/15). However screening for Bz at baseline was only done in 70% episodes (32/46) which were found negative.

Another grand review[4] of pathogenesis and treatment of HE in 1991 summarized the reported studies and found an amelioration of HE in 69% of patients and 76% of the episodes. Approximately 88% of those patients who did not respond to flumazenil were in stage 4, 60% had increased intracranial pressure (the authors didn't mention how to make this diagnosis) and 60% subsequently died with 3 days. This review included simultaneously both uncontrolled and controlled studies, HE of PSE and FHF, and clinical and electrophysiological improvement.

A recent open trial[49] (one part of the whole study) of 13 cases does not support a major therapeutic effect of flumazenil on HE, even most of the cases were Bz-screening positive, whom are considered to show positive reactions quite likely.

Several controlled trials have been conducted.

A placebo-controlled trial[32] reported in abstract form (1989) showed that flumazenil improved neurological status in 13 of 18 patients (72%), compared with 0% during the placebo period. However, it did not mention whether Bzs were prior used or had been screened.

In the second double-blind cross-over study[48] also published only in abstract form (1989), significant improvement with flumazenil over placebo could not be concluded in 8 FHF or cirrhotic patients with HE. It is the same investigation group that published another article[49] with the same design in 1995 (unfortunately, unavailable of the original paper, but only it abstract) which shows in the bolus period of 18 HE cases, 6 responded clinically to flumazenil while only 2 improved in the placebo period, with the p value of 0.06. However, the EEG grade did not change in any of the patients. Furthermore, there was no significant difference between the two groups during the infusion period. This study

concluded that it does not support a major therapeutic effect of flumazenil on HE.

Another small double-blind, placebo-controlled, randomized cross-over trial (1994) provided an important conclusion[45] that flumazenil may be a useful agent in improving the neurological condition of some patients with HE. It showed beneficial results, fast, distinct but transient, in a subset (40%) of cirrhotic patients with severe HE (Stage 4), hepatic coma. Moreover it showed that the efficacy of flumazenil in this study was not related to the presence or absence of the Bzs in the blood after screening the blood concentrations of Bzs of all the patients with both positive and negative results in both treatment and control phases. The evaluating method used in this research was modified Glasgow coma scale, which has been found not closely related to the clinical stages of HE.

The latest (1995) multicenter double-blind pragmatic randomized, placebo published study[39] demonstrated the effect of infusion of 0.4-1 mg flumazenil -- a modest but rapid (4-47 mins, mean 7 mins) improvement in the EEG grading of HE and a moderate but delayed (30-340 mins, mean 83 mins) improvement in the clinical grade of HE, while no favorable changes happened

in the placebo group ($p < 0.01$). However, it didn't mention the effect lasting time and the definition of clinical improvement clearly. This therapeutic result did not change with the inclusion or exclusion of positive Bz-screened patients.

Generally, most of these studies introduce flumazenil iv bolus administration, but with different dosages; some are conducted in cross-over design; the evaluation measures include clinical conditions and EEG stages, but with different definition of the "improvement" or "effectiveness". The effect lasting time of flumazenil, the potential or possible influence of flumazenil on the consequent outcome of the patients haven't been explained clearly in these articles.

Very few studies evaluated the survival of HE patients after flumazenil administration. Only one successful case of long-term treatment[36] with orally repeated administration of flumazenil has been reported. An un-controlled study with 14 cases[18] reported that 7 of the 10 flumazenil responders were ultimately discharged from the hospital and only 1 of the responders died of liver failure rather than failure of other organs. The researchers discussed the prognostic value of the flumazenil-induced arousal. Because 3 of the 4 non-

responders died within 2 days and 2 died of end-stage of renal failure and hepatic carcinoma besides hepatic coma, whether the response to flumazenil does indeed have any prognostic significance, or flumazenil does change the death rate of these HE patients remains to be established.

2.7 What Will This Research Do?

In general, currently available data suggest that BzR antagonists are likely to be an important adjunct to conventional therapies for HE. No doubt well-designed double-blind controlled studies comparing BzR antagonists to placebo and conventional therapies in patients with HE are necessary, better with the much larger sample size.

Flumazenil has been empirically used in the author's setting. Some positive reactions have been noted. However, since flumazenil is quite expensive considering its dosage (0.5 mg/ampoule/$45 in Shanghai) and its relatively short maintenance duration, the position of clinical practicability and acceptability in clinical service awaits to be proven. Though it may show somewhat dramatic improvement of mental status of some patients, which makes some doctors be very active in offering this agent, it is not recommended for this prescription in common until the acceptable results from

systematic controlled trials demonstrate the evidence. Moreover, the data from the clinical trials can be utilized for future economic evaluation on the perspectives of patients (and their relatives) and health providers. It may well be that an increase in the treatment resources including flumazenil and other therapies available to evaluate and treat HE patients would yield benefits, in terms of restored or prolonged productivity of the patients as well as the relatives, that would outweigh the cost[5,14].

Considering the characteristics of HE, the special property of flumazenil, and the practicability in the research setting, the cirrhotic, other than FHF, patients with HE will be enrolled, the mental status showing the clinical stage will be used as the primary tool[2] to grade the HE patients before and after the administration of flumazenil (1 mg iv bolus) in this study. The relevance of clinical changes are emphasized. The stage changes of these patients will be recorded to obtain the effect lasting time. The PSE Index will be also used but at fewer time-points to assist the evaluation of the effect of flumazenil and to assess the daily changes of the mental status of these patients after flumazenil administration.

Based on these figures, a hospital-based, prospective randomized double-blind controlled clinical trial is designed to evaluate the effectiveness of flumazenil in the treatment of cirrhotic patients with HE and the clinical courses of HE after flumazenil therapy until their discharge or death in the ward.